

*Analysis of the rib, iliac crest, vertebra, and sternum for fluoride indicates a direct increase in bone fluoride with an increase in fluoride in drinking water up to 4.0 ppm.*

## Fluoride deposition in human bones

*after prolonged ingestion*

*of fluoride in drinking water*

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**I**N CONJUNCTION with microscopic examination of human bones for possible effects of prolonged ingestion of fluoride in drinking water (reported in this issue by Geever and associates, pp. 721-731), essentially the same bones were analyzed for ash and fluoride. These bones were obtained at autopsy from 69 persons, 36 of whom were exposed to 1.0 to 4.0 ppm fluoride for 10 to 76 years. The remaining 33 had lived in areas with less than 1.0 ppm fluoride for 10 to 87 years.

This study represents the first systematic survey of the fluoride concentration of bones of individuals exposed to various concentrations of fluoride in drinking water. Previous studies of exposure to high levels of fluoride, recently reviewed by McClure and associates (pp. 741-746 of this issue), have dealt with no more than

four cases (1-5). More extensive data have been reported on the fluoride concentration of selected bones of individuals in low-fluoride areas (6-8).

Most of the persons whose bones were examined in the present studies died suddenly, the chief causes being trauma, coronary heart disease, and cerebrovascular accidents. Although bones of persons with chronic illness or diseases known to affect bone structure were excluded from the microscopic study, they were included in the fluoride deposition study so that the effect of such conditions might be observed. The fluoride analysis thus included bones from 3 individuals with a malignancy and 3 with renal disease.

The 69 individuals in the fluoride deposition study, 40 men and 29 women aged 26 through 90 years, provided 190 bones and 64 specimens of intervertebral cartilage. Approximately 80 percent of these bones and cartilage specimens were also examined histologically. The bones, consisting of a portion of the iliac crest, the lumbar vertebra, and the sixth rib, were fixed in 10 percent formalin at autopsy.

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**Table 1. Percentage ash and fluoride in dry, fat-free bones of individuals ingesting drinking water containing less than 1 ppm fluoride**

| Sub-<br>ject<br>No.           | Sex    | Age<br>(years) | Resi-<br>dence<br>(years) | Iliac crest |          | Rib  |          | Vertebra |          |
|-------------------------------|--------|----------------|---------------------------|-------------|----------|------|----------|----------|----------|
|                               |        |                |                           | Ash         | Fluoride | Ash  | Fluoride | Ash      | Fluoride |
| 0.1 ppm fluoride              |        |                |                           |             |          |      |          |          |          |
| 1                             | Male   | 27             | 27                        | 56.8        | 0.019    | 61.4 | 0.020    | 50.2     | 0.031    |
| 2                             | Male   | 32             | 12                        | 56.2        | .044     | 56.9 | .037     | 50.4     | .054     |
| 3                             | Male   | 35             | 35                        | 58.1        | .045     | 59.4 | .043     | 43.7     | .043     |
| 4                             | Female | 38             | 12                        |             |          |      |          | 52.2     | .024     |
| 5                             | Male   | 47             | 10                        |             |          |      |          | 49.9     | .056     |
| 6                             | Female | 47             | 15                        | 57.6        | .020     | 57.9 | .024     | 46.6     | .019     |
| 7                             | Male   | 50             | 25                        | 59.6        | .030     | 60.9 | .024     | 51.7     | .026     |
| 8                             | Female | 55             | 20                        | 58.9        | .038     | 53.9 | .043     | 43.8     | .036     |
| 9                             | Male   | 56             | 20                        | 52.6        | .046     | 57.8 | .047     | 47.9     | .057     |
| 10                            | Male   | 62             | 62                        | 54.9        | .032     | 57.6 | .028     | 50.9     | .038     |
| 11                            | Male   | 62             | 62                        | 58.8        | .024     | 57.3 | .031     | 46.8     | .028     |
| 12                            | Male   | 65             | 41                        | 54.2        | .034     | 54.0 | .062     | 43.2     | .034     |
| 13                            | Male   | 66             | 15                        | 57.6        | .028     | 61.9 | .039     | 45.7     | .026     |
| 14                            | Female | 68             | 10                        | 62.9        | .073     | 65.5 | .077     | 55.7     | .079     |
| 15                            | Male   | 69             | 10                        | 58.3        | .035     | 60.0 | .037     | 50.5     | .034     |
| 16                            | Male   | 70             | 60                        | 54.8        | .032     | 60.2 | .039     | 44.3     | .042     |
| 17                            | Female | 73             | 32                        | 54.0        | .048     | 57.6 | .060     | 44.6     | .055     |
| 18                            | Female | 82             | 40                        | 56.8        | .044     | 58.8 | .037     |          |          |
| 19                            | Male   | 87             | 87                        | 59.2        | .055     | 59.8 | .062     | 46.9     | .059     |
| 0.2 ppm fluoride              |        |                |                           |             |          |      |          |          |          |
| 20                            | Female | 32             | 10                        |             |          | 68.4 | 0.023    | 53.6     | 0.038    |
| 21                            | Male   | 32             | 32                        | 58.8        | 0.053    | 64.1 | .031     | 51.7     | .043     |
| 0.3 ppm fluoride <sup>2</sup> |        |                |                           |             |          |      |          |          |          |
| 22                            | Female | 48             | 48                        |             |          | 60.5 | 0.048    |          |          |
| 23                            | Male   | 53             | 24                        | 59.7        | 0.046    | 54.4 | .056     | 47.3     | 0.050    |
| 24                            | Male   | 59             | 57                        |             |          |      |          | 46.2     | .093     |
| 25                            | Female | 74             | 25                        | 59.0        | .078     | 57.9 | .089     | 43.7     | .077     |
| 0.1-0.4 ppm fluoride          |        |                |                           |             |          |      |          |          |          |
| 26                            | Male   | 32             | 32                        |             |          | 56.2 | 0.062    | 45.4     | 0.061    |
| 27                            | Female | 40             | 10                        |             |          | 60.6 | .071     | 49.8     | .111     |
| 28                            | Male   | 44             | 10                        |             |          | 53.4 | .039     | 46.4     | .042     |
| 29                            | Male   | 57             | 34                        |             |          | 60.2 | .039     | 44.9     | .040     |
| 30                            | Male   | 59             | 10                        |             |          | 54.4 | .078     | 48.5     | .094     |
| 31                            | Male   | 64             | 10                        |             |          | 60.4 | .064     | 51.4     | .075     |
| 32                            | Male   | 71             | 10                        |             |          | 60.0 | .069     | 45.3     | .083     |
| 33                            | Male   | 74             | 20                        |             |          | 51.3 | .106     | 49.8     | .119     |
| Mean                          |        | 55.5           | 28.1                      | 57.4        | .041     | 58.8 | .050     | 49.0     | .054     |
| SE                            |        | 2.8            | 3.4                       | .3          | .003     | .7   | .004     | .6       | .005     |

<sup>1</sup> Resident of Baltimore, Md.; water fluoridated to 1 ppm in November 1952; received fluoridated water for 2 years.

<sup>2</sup> Residents of Washington, D. C.; water fluoridated to 1 ppm in June 1952; received fluoridated water 4, 2, 3, and 3 years, respectively.

The intervertebral cartilage was separated from the vertebra, and all specimens were cleaned of adhering soft tissues. Each sample was dried overnight at 105° C., broken into pieces, and extracted with alcohol for 8 hours and with ether for 4 hours. After the sample was ground to pass a 60-mesh sieve, a portion was ashed for 3 hours at 550° C. and analyzed for fluoride (9-11). The significance of the differences in means was calculated according to Fisher's *t* test for paired values. The sodium, potassium, calcium, magnesium, phosphorus, carbon dioxide, and citrate contents of these tissues will be reported later.

### Bone Fluoride

The percentages of ash and fluoride in dry, fat-free bones for each individual in the study, grouped according to the level of fluoride in the drinking water, are recorded in tables 1 through 4. Ages and years of residence are also given, but it is not possible to detect any definite relation between the fluoride concentration and these factors.

The fluoride values for the 15 bones from the individuals who had a malignancy or renal disease were within the variations encountered for others in the same group (subject No. 5 in table 1; subjects Nos. 8, 10, and 11 in table 3; iliac crest, subject No. 1 in table 4) or slightly higher (subject No. 2 in table 2; rib and vertebra, subject No. 1 in table 4). No significance can be attached to the somewhat higher values since they occurred in individuals from the two

smaller groups (tables 2 and 4). Moreover, approximately a tenfold increase in fluoride concentration of the bones from the lowest to the highest fluoride areas (tables 1 and 4) was seen without any apparent tissue damage.

### Less Than 1 ppm Fluoride

Individuals exposed to less than 1 ppm fluoride were residents of several localities, with fluoride concentrations in the drinking water varying from 0.1 ppm in New York City to 0.4 ppm in San Antonio, Tex. The mean was 0.2 ppm. Five persons in this group, aged 32 through 74 years, drank water fluoridated to 1 ppm fluoride for 2 to 4 years. The fluoride content of the bones of these five was within the variations encountered in other samples in this group (table 1).

On a dry, fat-free basis, the mean concentrations of fluoride in the various bones of individuals exposed to less than 1 ppm fluoride were similar, 0.041, 0.050, and 0.054 percent for the iliac crest, the rib, and the vertebra, respectively. As expected, the fluoride concentrations of the rib and the vertebra were generally highest in the individuals from San Antonio.

Five samples of sternum from this group varied from 0.040 to 0.101 percent fluoride with a mean,  $\pm$  standard error, of  $0.066 \pm 0.012$  percent (not including one inordinately high value of 0.229 percent fluoride). On an ash basis, they ranged from 0.078 to 0.188 with a mean of 0.134 percent. The mean concentration of fluoride in the ash of the vertebra ( $0.112 \pm 0.010$

**Table 2. Percentage ash and fluoride in dry, fat-free bones of individuals ingesting drinking water containing 1 ppm fluoride, Grand Rapids, Mich.<sup>1</sup>**

| Subject No. | Sex    | Age (years) | Residence (years) | Iliac crest |          | Rib  |          | Vertebra |          |
|-------------|--------|-------------|-------------------|-------------|----------|------|----------|----------|----------|
|             |        |             |                   | Ash         | Fluoride | Ash  | Fluoride | Ash      | Fluoride |
| 1           | Female | 64          | 12                |             |          | 57.8 | 0.135    | 45.9     | 0.130    |
| 2           | Female | 64          | 15                | 61.9        | 0.176    | 59.7 | .195     | 48.4     | .238     |
| 3           | Male   | 82          | 10                | 59.5        | .137     | 54.5 | .119     | 51.3     | .140     |
| 4           | Female | 83          | 20                | 61.1        | .137     |      |          | 53.3     | .135     |
| 5           | Male   | 85          | 50                | 62.2        | .100     | 57.4 | .106     | 50.9     | .159     |
|             | Mean   | 75.6        | 21.5              | 61.2        | .138     | 57.4 | .139     | 50.0     | .160     |
|             | SE     | 1.5         | 7.4               | .6          | .016     | 1.1  | .020     | 1.3      | .020     |

<sup>1</sup> Fluoride added to drinking water January 1945. Subjects received fluoridated water for 12 years with exception of male aged 82 years.

percent) was similar to that of the sternum ash ( $0.134 \pm 0.023$  percent), but it was significantly higher than that in the ash of the iliac crest ( $0.072 \pm 0.006$  percent,  $P < 0.01$ ) or in the rib ( $0.086 \pm 0.008$  percent,  $P < 0.05$ ).

While ash concentrations of the vertebra and the sternum were similar (49.0 percent and 49.4 percent), they were significantly lower than that of the iliac crest (57.4 percent,  $P < 0.01$ ) or the rib (58.8 percent,  $P < 0.01$ ).

#### *Fluoride Level of 1 ppm*

A second group, composed of 5 persons, were residents of Grand Rapids, Mich., where the water supply was fluoridated to 1 ppm in January 1945. The autopsy specimens were obtained between November 1956 and January 1957; thus exposure to 1 ppm fluoride was for a maximum of 12 years during the last years of life.

As with the low-fluoride group, no differences were found in the concentration of fluo-

ride in the various bones of this group (table 2). On a dry, fat-free basis, the mean percentages were 0.138 for the iliac crest, 0.139 for the rib, and 0.160 for the vertebra. The fluoride concentrations of the ash of the iliac crest ( $0.225 \pm 0.026$  percent) and of the rib ( $0.241 \pm 0.030$  percent) were similar, but they were significantly lower than that of the vertebra ( $0.323 \pm 0.043$  percent,  $P < 0.05$ ). Appreciable amounts of fluoride thus accrued during the last 10 to 12 years of life of these 5 individuals aged 64 through 85 years.

For the 5 exposed to 1 ppm fluoride, the concentrations of ash in the iliac crest (61.2 percent) and the rib (57.4 percent) were similar but significantly higher than that of the vertebra (50.0 percent,  $P < 0.01$ ).

#### *Fluoride Level of 2.6 ppm*

Colorado Springs, Colo., and Amarillo, Tex., were the residences of 27 subjects. Colorado Springs has had a uniform and verified history

**Table 3. Percentage ash and fluoride in dry, fat-free bones of individuals ingesting drinking water containing a mean of 2.6 ppm fluoride, Colorado Springs, Colo., and Amarillo, Tex.**

| Sub-<br>ject<br>No. | Sex    | Age<br>(years) | Resi-<br>dence<br>(years) | Iliac crest |          | Rib  |          | Vertebra |          |
|---------------------|--------|----------------|---------------------------|-------------|----------|------|----------|----------|----------|
|                     |        |                |                           | Ash         | Fluoride | Ash  | Fluoride | Ash      | Fluoride |
| 1                   | Male   | 36             | 10                        | 59.6        | 0.095    | 57.8 | 0.106    | 47.3     | 0.098    |
| 2                   | Male   | 36             | 36                        | 58.9        | .092     | 61.1 | .103     | 49.7     | .145     |
| 3                   | Female | 47             | 10                        | 57.3        | .219     | 59.7 | .194     | 54.3     | .239     |
| 4                   | Female | 47             | 15                        | 61.4        | .481     | 66.7 | .370     | 56.8     | .521     |
| 5                   | Male   | 50             | 10                        | 57.9        | .129     | 58.3 | .199     | 52.7     | .179     |
| 6                   | Female | 53             | 22                        | 61.2        | .111     | 62.2 | .134     | 52.4     | .163     |
| 7                   | Female | 54             | 20                        | 62.0        | .247     | 65.0 | .179     | 53.8     | .332     |
| 8                   | Male   | 54             | 20                        |             |          | 56.1 | .269     | 43.7     | .325     |
| 9                   | Male   | 56             | 10                        | 57.3        | .141     | 55.1 | .104     | 44.9     | .150     |
| 10                  | Male   | 57             | 46                        | 58.2        | .288     | 59.5 | .278     | 32.7     | .197     |
| 11                  | Male   | 58             | 25                        |             |          | 58.9 | .391     | 45.0     | .334     |
| 12                  | Female | 63             | 25                        | 60.0        | .297     | 58.0 | .279     | 47.8     | .305     |
| 13                  | Male   | 65             | 30                        | 60.0        | .268     | 56.9 | .276     | 47.9     | .299     |
| 14                  | Female | 66             | 10                        | 62.7        | .214     | 59.4 | .194     | 44.3     | .244     |
| 15                  | Female | 69             | 28                        | 59.9        | .458     | 58.2 | .453     | 50.7     | .548     |
| 16                  | Male   | 71             | 36                        | 53.3        | .454     | 57.2 | .363     | 47.2     | .400     |
| 17                  | Female | 75             | 16                        | 60.8        | .237     | 59.3 | .244     | 42.6     | .262     |
| 18                  | Male   | 76             | 76                        | 61.6        | .371     | 55.5 | .347     | 54.0     | .346     |
| 19                  | Male   | 79             | 40                        | 61.7        | .176     | 59.4 | .161     | 47.3     | .189     |
| 20                  | Female | 80             | 13                        | 60.4        | .190     | 59.5 | .205     | 50.2     | .293     |
| 21                  | Female | 81             | 52                        | 60.1        | .321     | 59.3 | .293     | 36.6     | .264     |
| 22                  | Female | 82             | 76                        | 56.4        | .334     | 52.3 | .338     | 46.0     | .382     |
| 23                  | Female | 83             | 54                        | 62.0        | .325     | 62.4 | .267     | 51.9     | .367     |
| 24                  | Female | 83             | 60                        | 58.6        | .348     | 62.4 | .373     | 56.1     | .363     |
| 25                  | Male   | 84             | 32                        | 60.9        | .154     | 58.6 | .121     | 53.7     | .177     |
| 26                  | Female | 84             | 62                        | 59.3        | .431     | 61.7 | .443     | 45.4     | .321     |
| 27                  | Male   | 90             | 55                        | 60.9        | .304     | 62.2 | .245     | 48.1     | .295     |
| Mean                |        | 65.9           | 32.9                      | 59.7        | .267     | 59.7 | .257     | 48.3     | .286     |
| SE                  |        | 3.0            | 4.0                       | .4          | .023     | .6   | .020     | 1.1      | .023     |

**Table 4. Percentage ash and fluoride in dry, fat-free bones of individuals ingesting drinking water containing a mean of 4.0 ppm fluoride, Lubbock, Tex.**

| Subject No. | Sex         | Age (years) | Residence (years) | Iliac crest |          | Rib  |          | Vertebra |          |
|-------------|-------------|-------------|-------------------|-------------|----------|------|----------|----------|----------|
|             |             |             |                   | Ash         | Fluoride | Ash  | Fluoride | Ash      | Fluoride |
| 1           | Male.....   | 26          | 17                | 58.6        | 0.445    | 62.8 | 0.458    | 51.2     | 0.564    |
| 2           | Male.....   | 53          | 10                | 60.8        | .374     | 54.5 | .401     | 47.5     | .348     |
| 3           | Male.....   | 71          | 48                | 62.1        | .542     | 53.3 | .449     | 59.9     | .470     |
| 4           | Female..... | 74          | 10                | 58.1        | .291     | 59.4 | .284     | 44.3     | .261     |
|             | Mean.....   | 56          | 21.3              | 59.9        | .413     | 57.5 | .398     | 50.7     | .411     |
|             | SE.....     | 11          | 9.1               | .9          | .053     | 2.2  | .040     | 3.4      | .068     |

of 2.5 ppm fluoride within narrow limits for some 75 years (12, 13). The fluoride level of the water of Amarillo from 1934 to 1948 ranged from 3.6 ppm to 6.2 ppm (14). According to a personal communication from the city chemist, 33 new wells were added between 1948 and 1953, and the fluoride level ranged from 2.6 to 3.2 ppm. In 1955 and 1956 the water supply of Carson County, which probably contains less than 1 ppm fluoride inasmuch as Dean (15) considered the incidence of mottled enamel virtually negative, was developed to augment that of Amarillo. In 1957, 92 wells were pumping into the Amarillo reservoir, and the fluoride content averaged 1.6 ppm. From 1948 to 1957, the fluoride level of the drinking water in Amarillo averaged 2.8 ppm, and the weighted mean for the two cities was 2.6 ppm.

For the 27 individuals, the mean fluoride concentration, on a dry, fat-free basis, of the iliac crest was 0.267 percent; of the rib, 0.257 percent; and of the vertebra, 0.286 percent (table 3). On an ash basis, the mean concentration of fluoride in the vertebra ( $0.598 \pm 0.015$  percent) was significantly higher than that in the iliac crest ( $0.450 \pm 0.040$  percent,  $P < 0.01$ ) or in the rib ( $0.432 \pm 0.036$  percent,  $P < 0.01$ ). The concentration of ash in the iliac crest and the rib (59.7 percent) was significantly higher than that in the vertebra (48.3 percent,  $P < 0.01$ ).

#### *Fluoride Level of 4.0 ppm*

Prolonged exposure to about 4.0 ppm fluoride in the drinking water took place in Lubbock, Tex., now the largest community in the United States reported to have this much fluoride. Because of the unusually high fluoride content of

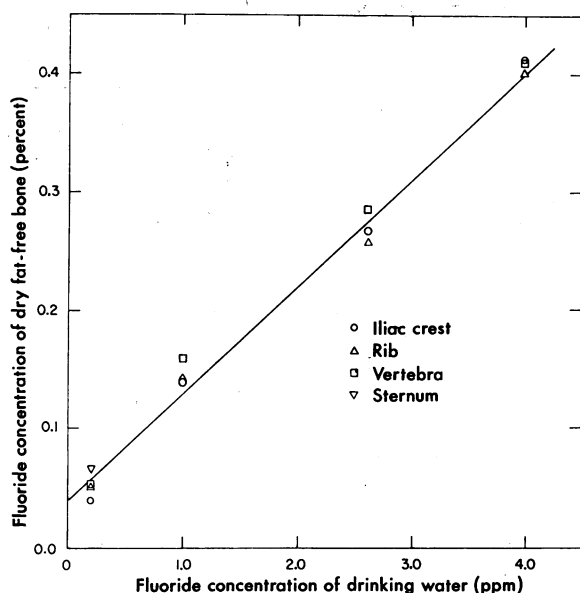
its drinking water, Lubbock, as well as Amarillo, has been the scene of a number of previous studies of the effects of fluoride (14-18). The water fluoride of Lubbock up to 1948 ranged from 3.5 to 4.4 ppm (14). In a recent letter from the Lubbock City-County Health Department, the fluoride content of the water supply from storage reservoirs was reported to have varied from 3.1 to 5.2 ppm from 1948 through 1955, with a mean of 4.0 ppm. The four subjects from Lubbock were autopsied from May through July 1955.

For the Lubbock residents as for the other subjects, the fluoride concentrations of the dry, fat-free iliac crest, rib, and vertebra were not significantly different, being 0.413, 0.398, and 0.411 percent, respectively. Although the concentration of fluoride in the ash of the vertebra ( $0.802 \pm 0.109$  percent) was greater than that of the iliac crest ( $0.687 \pm 0.081$  percent) or that of the rib ( $0.629 \pm 0.095$  percent), the difference was not significant ( $P > 0.05$ ). The ash content of the iliac crest (59.9 percent) and that of the rib (57.5 percent) were similar but higher than the ash content of the vertebra (50.7 percent). An unusually high value of 59.9 percent ash for a specimen of vertebra prevented any level of significance being attached to the difference in the mean ash content of the various bones.

#### *Group Comparison*

To summarize these data, the mean concentrations of fluoride in the various bones were plotted against the fluoride level of the drinking water to which the individuals were exposed (see chart). The result indicates that the relation between fluoride in the bones and fluoride

# **Relation of fluoride in dry, fat-free bones to fluoride in drinking water.**



in the drinking water up to 4.0 ppm is adequately described by a straight line function.

## **Other Findings**

The concentration of fluoride in the dry, fat-free intervertebral cartilage also appeared to increase somewhat with elevated levels of water-borne fluoride (table 5). Calcification of this tissue, however, appears not to have been affected by the fluoride concentration of the drinking water.

In addition to the fluoride and ash analyses reported in the preceding tables, it may be of interest to note that the fat content of neither the bones nor the intervertebral cartilage was

related to the fluoride content of the drinking water. The mean concentrations of fat in the oven-dry bones had the following range:

|                             | Fat content (percent) |            |
|-----------------------------|-----------------------|------------|
|                             | Lowest                | Highest    |
| Iliac crest.....            | 23. 2±5. 7            | 35. 3±2. 6 |
| Rib.....                    | 23. 4±3. 6            | 30. 2±5. 7 |
| Vertebra.....               | 32. 2±7. 6            | 43. 8±4. 3 |
| Sternum <sup>1</sup> .....  | 41. 3±4. 3            | 41. 3±4. 3 |
| Intervertebral cartilage... | 1. 8±. 2              | 3. 3±. 8   |

<sup>1</sup> Only one fluoride group examined.

## **Discussion**

The fluoride concentrations in the bones examined in the present study from both low-fluoride areas and high-fluoride areas approximate those observed in the previous limited studies (tables 6 and 7). For one resident of London, a low-fluoride area, a very high value of 0.687 percent fluoride in rib ash was obtained (7). The highest concentration of fluoride found in the present study in bones of individuals who had been drinking water containing less than 1.0 ppm fluoride was 0.428 percent in a sample of ashed sternum, and the next high value was 0.238 percent fluoride for a sample of vertebral ash. In considering the data for high-fluoride areas, some reservations must be attached to the high values presented by Kilborn and associates (5), since these results may have been due to the subnormal living standards and the low nutritional status of this area (Chengtu, China).

Fluoride in the drinking water up to 4.0 ppm did not affect the ash content of any of the bones analyzed.

For any given level of fluoride in the drinking water, the mean concentrations of fluoride in the

**Table 5. Percentage ash and fluoride in intervertebral cartilage in relation to fluoride level of drinking water**

| Fluoride level of water (ppm) | Number of samples | Dry, fat-free basis |               | Ash basis     |
|-------------------------------|-------------------|---------------------|---------------|---------------|
|                               |                   | Ash                 | Fluoride      | Fluoride      |
| <1.0.....                     | 29                | 4. 8±0. 3           | 0. 003±0. 000 | 0. 049±0. 008 |
| 1.0.....                      | 5                 | 3. 9±. 3            | . 004±. 001   | . 090±. 029   |
| 2.6.....                      | 26                | 3. 8±. 2            | . 004±. 001   | . 098±. 015   |
| 4.0.....                      | 4                 | 4. 2±. 1            | . 007±. 003   | . 159±. 056   |

NOTE: Data are expressed as mean±standard error.

**Table 6. Percentage fluoride in human bone ash from low-fluoride areas**

| Fluoride level of water (ppm)           | Bone         | Number of samples | Fluoride in bone ash (percent) | Source of data            |
|---|--------------|-------------------|--------------------------------|---------------------------|
| 0.0                                     | Rib          | 1                 | 0.01                           | Boissevain and Drea (3).  |
|   | Humerus      | 1                 | .1                             | Do.                       |
|   | Tibia        | 1                 | .02                            | Do.                       |
|   | "Toe"        | 1                 | .16                            | Do.                       |
| Not given, presumably low. <sup>1</sup> | Skull        | 1                 | .059                           | Klement (19).             |
|   | "Long bones" | (2)               | .070                           | Do.                       |
|   | Rib          | 11                | 0.048-.21                      | Roholm (1).               |
|   | Iliac crest  | 13                | <sup>3</sup> .028-.128         | Martin (8).               |
| 0.1                                     | Rib          | 83                | .006-.252                      | Smith and associates (7). |
|   | Vertebra     | 83                | .005-.331                      | Do.                       |
| 0.0-0.5                                 | Rib          | 25                | .115-.687                      | Glock and associates (6). |
|   | Iliac crest  | 20                | .034-.132                      | Present study.            |
| 1.0                                     | Rib          | 30                | .033-.207                      | Do.                       |
|   | Vertebra     | 31                | .040-.238                      | Do.                       |
|   | Sternum      | 5                 | .078-.188                      | Do.                       |

<sup>1</sup> Designated as "normal" individuals.<sup>2</sup> Not given.<sup>3</sup> Data originally given on dry, fat-free basis; calculated to ash basis assuming mean ash value of 59.0 percent for iliac crest from present study.**Table 7. Percentage fluoride in human bone ash from high-fluoride areas**

| Fluoride level of water (ppm) | Bone          | Number of samples | Fluoride in bone ash (percent) | Source of data                       |
|-------------------------------|---------------|-------------------|--------------------------------|--------------------------------------|
| 2.0                           | Rib           | 4                 | 0.12-0.35                      | Boissevain and Drea (3)              |
| 5.9 and 6.3                   | Miscellaneous | 15                | 1.47-2.21                      | Kilborn and associates (5)           |
|                               | Sternum       | 1                 | .69                            | Linsman and McMurray (4).            |
| 4.4-12.0                      | Vertebra      | 1                 | .75                            | Do.                                  |
| 8.0                           | Miscellaneous | 8                 | .715-.967                      | McClure and associates. <sup>1</sup> |
| 1.0-4.0                       | Iliac crest   | 33                | .157-.872                      | Present study.                       |
|                               | Rib           | 35                | .150-.843                      | Do.                                  |
|                               | Vertebra      | 36                | .208-1.103                     | Do.                                  |

<sup>1</sup> This issue of *Public Health Reports*, pp. 741-746.

dry, fat-free iliac crest, rib, and vertebra were similar. On an ash basis, however, the fluoride concentration of the vertebra was significantly higher than that of the rib at fluoride levels up to 2.6 ppm. At higher amounts of fluoride in the drinking water, this difference did not obtain.

Previous studies dealing with fluoride deposition in the white rat (20-22) and urinary fluoride excretion by the rat (23) and by man (24, 25) have indicated that as bone ages it may lose some of its capacity to store fluoride. This decrease in fluoride deposition with increasing age has been presented in graphic form by Hodge (26) based on the data on man of Smith and associates (7). In the present study, most of the subjects were of advanced age, and the

means for the four groups varied only from 55.5 to 75.6 years. Hence, on the one hand, it was unlikely that a relation between fluoride deposition and age would be apparent; on the other hand, the age factor would tend not to bias any relation between bone fluoride and water fluoride.

From this extensive survey on the fluoride content of human skeletal tissues, it appears that the deposition of fluoride is directly related to the fluoride content of the drinking water up to 4.0 ppm. The deposition of fluoride in dentin and enamel is also elevated proportionately with an increasing concentration of fluoride in the drinking water (27).

There is no indication in these data or in those of previous studies that these human calcified

tissues approach their theoretical capacity of about 3.5 percent fluoride, although drinking water containing as much as 4.0 ppm fluoride was ingested. Prolonged exposure to the water of Colorado Springs, Colo., and Amarillo, Tex. (2.6 ppm fluoride), and to that of Lubbock, Tex. (4.0 ppm fluoride), elevated the fluoride content of the bone ash to a maximum of 1.080 and 1.103 percent, respectively.

The data from the five subjects of Grand Rapids, Mich., are of special interest. Exposure to water containing 1 ppm fluoride did not exceed 12 years, and the average age of the subjects at the time they started to use fluoridated water was about 63 years. Nevertheless, a mean concentration of 0.146 percent fluoride on a dry, fat-free basis was found in these relatively old bones, compared with an average value of 0.049 percent in the bones of individuals of an average age of 56 years ingesting water containing less than 1.0 ppm fluoride.

The fluoride data in the present study coupled with the microscopic findings on essentially the same individuals constitute substantial evidence that a concentration of fluoride as high as 0.548 percent in the dry, fat-free bone and 1.080 percent in the bone ash (for subjects in both studies) may be present without producing any apparent tissue damage.

### Summary

Selected bones and skeletal tissue—iliac crest, rib, vertebra, sternum, and intervertebral cartilage—obtained from 69 individuals 26 through 90 years of age who had drunk water containing 0.1 to 4.0 ppm fluoride for at least 10 years were analyzed for ash and fluoride. These bones, which showed no significant histological changes, contained up to 0.548 percent fluoride on a dry, fat-free basis and 1.080 percent fluoride on an ash basis. The mean ash content of the iliac crest and ribs varied from 57.4 to 61.2 percent and that of the sternum and vertebra from 44.6 to 53.7 percent.

At any given level of fluoride in the drinking water, the various bones contained, on a dry, fat-free basis, similar concentrations of fluoride. The fluoride concentration of the intervertebral cartilage was considerably less than that of the bones.

The concentration of fluoride in the bones increased in an essentially linear fashion with an increase of fluoride in the drinking water up to 4.0 ppm.

### REFERENCES

- (1) Roholm, K.: Fluorine intoxication. A clinical-hygienic study. London, H. K. Lewis, 1937.
- (2) Wolff, W. A., and Kerr, E. G.: The composition of human bone in chronic fluoride poisoning. *Am. J. M. Sc.* 195: 493-497, April 1938.
- (3) Boissevain, C. H., and Drea, W. F.: Spectroscopic determination of fluorine in bones and teeth and other organs in relation to fluorine in drinking water. *J. Dent. Res.* 13: 495-500, December 1933.
- (4) Linsman, J. F., and McMurray, C. A.: Fluoride osteosclerosis from drinking water. *Radiology* 40: 474-484, May 1943.
- (5) Kilborn, L. G., Outerbridge, T. S., and Lei, H.-P.: Fluorosis with report of an advanced case. *Canad. M. A. J.* 62: 135-141, February 1950.
- (6) Glock, G. E., Lowater, F., and Murray, M. M.: The retention and elimination of fluorine in bones. *Biochem. J.* 35: 1235-1239, November 1941.
- (7) Smith, F. A., Gardner, D. E., and Hodge, H. C.: Skeletal storage of fluorine in the human. Atomic Energy Commission Quarterly Technical Report UR-200. Washington, D. C., 1952, pp. 12-14.
- (8) Martin, D. J.: The Evanston dental caries study. I. Determination of fluorine in foods, bones and teeth. *J. Dent. Res.* 27: 27-33, February 1948.
- (9) Willard, H. H., and Winter, O. B.: Volumetric method for determination of fluorine. *Indust. & Engin. Chem. (Analyt. Ed.)* 5: 7-10, January 1933.
- (10) Armstrong, W. D.: Microdetermination of fluorine. Elimination of effect of chloride. *Indust. & Engin. Chem. (Analyt. Ed.)* 8: 384-387, September 1936.
- (11) McClure, F. J.: Microdetermination of fluorine by thorium nitrate titration. *Indust. & Engin. Chem. (Analyt. Ed.)* 11: 171-173, March 1939.
- (12) Dean, H. T., Arnold, F. A., Jr., and Elvove, E.: Domestic water and dental caries. V. Additional studies of the relation of fluoride domestic waters to dental caries experience in 4,425 white children, aged 12 to 14 years of 13 cities in 4 States. *Pub. Health Rep.* 57: 1155-1179, Aug. 7, 1942.
- (13) Russell, A. L., and Elvove, E.: Domestic water and dental caries. VII. A study of the fluoride-dental caries relationship in an adult population. *Pub. Health Rep.* 66: 1389-1401, Oct. 26, 1951.



- (14) McCauley, H. B., and McClure, F. J.: Effect of fluoride in drinking water on the osseous development of the hand and wrist in children. *Pub. Health Rep.* 69: 671-683, July 1954.
- (15) Dean, H. T.: Distribution of mottled enamel in the United States. *Pub. Health Rep.* 48: 703-734, June 23, 1933.
- (16) Dean, H. T., Dixon, R. M., and Cohen, C.: Mottled enamel in Texas. *Pub. Health Rep.* 50: 424-442, Mar. 29, 1935.
- (17) McClure, F. J., and Kinser, C. A.: Domestic waters and systemic effects. II. Fluorine content of urine in relation to fluorine in drinking water. *Pub. Health Rep.* 59: 1575-1591, Dec. 8, 1944.
- (18) McClure, F. J.: Fluoride domestic waters and systemic effects. I. Relation to bone-fracture experience, height, and weight of high school boys and young selectees of the Armed Forces of the United States. *Pub. Health Rep.* 59: 1543-1558, Dec. 1, 1944.
- (19) Klement, R.: Der Fluorgehalt der Knochen und Zähne. *Ber. d. deutsch. chem. Gesellsch.* 69: 2012-2019, November 1935.
- (20) Zipkin, I., and McClure, F. J.: Deposition of fluorine in the bones and teeth of the growing rat. *J. Nutrition* 47: 611-620, August 1952.
- (21) Savchuck, W. B., and Armstrong, W. D.: Metabolic turnover of fluoride by the skeleton of the rat. *J. Biol. Chem.* 193: 575-585, December 1951.
- (22) Jackson, S. H.: The stabilization of the fluorine concentration of the total ash of rats. *Canad. J. Biochem. & Physiol.* 33: 93-98, January 1955.
- (23) Laurenz, M., Mitchell, H. H., and Ruth, W. A.: Adaptation of the growing rat to the ingestion of a constant concentration of fluorine in the diet. *J. Nutrition* 19: 531-546, June 1940.
- (24) Zipkin, I., Likins, R. C., McClure, F. J., and Steere, A. C.: Urinary fluoride levels associated with the use of fluoridated waters. *Pub. Health Rep.* 71: 767-772, August 1956.
- (25) Zipkin, I., and Leone, N. C.: Rate of urinary fluoride output in normal adults. *Am. J. Pub. Health* 47: 848-851, July 1957.
- (26) Hodge, H. C.: The significance of the skeletal storage of fluoride. In *Tr. 4th Conf., Metabolic Interrelations With Special Reference to Calcium*. Caldwell, N. J., Progress Associates, Inc., January 1952, pp. 250-260.
- (27) McClure, F. J., and Likins, R. C.: Fluorine in human teeth in relation to fluorine in the drinking water. *J. Dent. Res.* 30: 172-176, April 1951.

## Training in Radiological Health

**Sanitary Engineering Center.** A course in the medical and biological aspects of radiological health will be given at the Robert A. Taft Sanitary Engineering Center, Public Health Service, October 20-31, 1958. Designed for medical, dental, and biological personnel responsible for program decisions in Federal, State, and local health agencies, the course will present data pertinent to radiological health program planning, with emphasis on radiation exposure in the healing arts.

Areas to be covered include biological effects of radiation, philosophy and procedures of radiation protection, practical methods of reducing exposure from medical and dental X-rays, administrative problems of a radiological health program, and the current status of legislative and regulatory procedures.

No previous formal training in radioactivity or radiation is necessary, and there are no tuition or fee charges. Application forms and further information concerning the course may be obtained from the Robert A. Taft Sanitary

Engineering Center, 4676 Columbia Parkway, Cincinnati 26, Ohio.

**University of Pittsburgh.** Beginning in 1958-59, 2- and 3-year courses in the health aspects of nuclear technology leading to master's and doctor's degrees will be offered by the Graduate School of Public Health. Although focused on occupational and health aspects of nuclear reactors, the new program will cover the fields of health physics and radiation biology. In addition to graduate school facilities, other University Medical Center facilities and some community installations will be used.

Pittsburgh's radiation health training program, located in the department of occupational health, was made possible by a 10-year grant from the Rockefeller Foundation. An additional grant of \$30,000 awarded by the Atomic Energy Commission will be used for equipment and instruments.

Applications and more information may be obtained from the Secretary, Graduate School of Public Health, University of Pittsburgh, Pittsburgh 13, Pa.